Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXII *APRIL, 1943* NUMBER 4
CONSECUTIVE No. 7

Pharmaceutical Progress as Reflected by the New Pharmacopoeia and Formulary

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Today the important measure of the value of any institution is its contribution to the war effort in its greater aspect. Standards which are more than mere specifications do make such a contribution.

Pharmaceutical standards, as represented by the United States Pharmacopœia and the National Formulary, are of particular value because they are standards which have been forged, not upon the anvils of emergent haste but which have been developed by continuous and arduous study and the tireless devotion of the best experts of the times to find a permanent place in our social structure. Pharmacy may note with pride that the need for standards which are dynamic and ever striving for perfection and maturity was appreciated as early as 1820 when the first United States Pharmacopœia was published as "being evidently the only mode by which a uniform system could be introduced into all parts of the American territory" because "in the United States the evil of irregularity and uncertainty in the preparation of medicines has been felt with a peculiar weight." It is because men from 1820 to the present day have never lost sight of their responsibility to attempt to fill this need that the Pharmacopœia and the National Formulary are in an advantageous position to contribute so greatly to the winning of the war.

It is the fact that a United States Pharmacopœia and a National Formulary exist which facilitates the interchange of medicinal agents with our Allies. Can you picture the chaotic and frenzied situation which would confront the agents of China, Britain or Russia if detailed information did not already exist as to the permissive tolerance of lead and arsenic in pectin, the manner in which insulin is standardized or the degree of purity of sulfathiazole? A simple problem would become needlessly complex, if upon seeking competitive bids for epinephrine, the Supply Missions were forced to compare standards upon many different bases. It is true, of course, that standards do differ from country to country, but the fact that these well-publicized criteria of identity and purity exist here permits a minimum of confusion.

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This value of having time-tried standards applies also to the needs of our armed forces. If such standards did not exist, much valuable time would be wasted in assembling information from which to prepare adequate specifications. It is extremely self-satisfying to learn how convenient the armed forces have found our standards and amazing to learn that many items, apt to be considered relatively unimportant, are now being manufactured by the ton in compliance with the standards of our official compendia.

But this is a war also being fought on the front of human relations and here again these books have proved themselves timely. For three decades the Pharmacopœia has been translated into Spanish, an act which is doubly significant now that the translation has been done under the jurisdiction of the Pan-American Sanitary Bureau, thus increasing its prestige in all Spanish-speaking nations. The "Pharmacopœia and the Physician" series has also been translated into Spanish and thus the Pharmacopœia and its agencies bid to be one of the strongest of the links in the chain of culture which now binds the Americas so intimately. The publication of a table of comparative Spanish and English titles in the U. S. P. XII should aid materially in the business and professional relations with our Spanish-speaking allies and friends.

All during the preparation of U. S. P. XII, the closest ties with the British Pharmacopœia authorities were maintained. Reports from both bodies were freely exchanged and collaborative research was undertaken. In addition, U. S. P. Reference Standards were based upon collaborative work with the Canadian Government which took cognizance of these standards in their enforcement programs.

Both the Pharmacopœia and the National Formulary have had machinery for some time for making Interim Revisions in order to facilitate their professional and judicial responsibilities. This machinery has been of great value to the war effort since it provides a relatively quick way of revising standards without lowering them as crucial materials become "tight." The permission to replace Oil of Lavender by Oil of Cedar Leaf, the modification of the storage requirements of Ergot on the part of the Pharmacopœia and the replacement of Belladonna by Strammonium in Hinkle's Pills by the National Formulary may be cited as examples of the expediency of this process.

The change from decennial to quinquennial revision not only means that the historical development of pharmaceutical standards will be measured in units of five years instead of ten, but it means rapid progress toward determining specifications for the medical supply table of the armed forces and an increased protection of the public as well. Realizing that the public invests over seven hundred million dollars annually in drugs and medicines and that less than one-third of this is upon the express order of physicians, the frequent revision of these books is wise. With the almost fantastically rapid advances being made in therapeutics as a result of martial demands, the five-year revision plan has become a necessity and it is a tribute to the progressiveness of democratic minds that provision anticipated necessity.

But let us look at the progress which has been made in drug standards during the last decade as reflected in these volumes, exclusive of values measured in terms of direct war contributions. We do well to evaluate the present Pharmacopœia with the stated aims of the First Pharmacopœia as the criterion. It was here stated that the careful selection of drugs which were most fully established and best understood and the convenient naming of such medicating agents are the objective of a Pharmacopœia. Furthermore, "The value of a Pharmacopœia depends upon the fidelity with which it conforms to the best state of medical knowledge of the day. Its usefulness depends upon the sanction it receives from the medical community and the public; and the extent to which it governs the language and practice of those for whose use it is intended."

Although complete specificity in medication has never been attained, we are closer to the goal, a factor which has meant a different emphasis upon therapeutic needs. A glance at the complete list of new admis-

sions to the Pharmacopœia shows that the Pharmacopœia "conforms to the best state of the medical knowledge of the day." Such important recent drugs as insulin, diphenylhydantoin sodium, sulfathiazole and human convalescent serum, to mention but a few, are now official and have been standardized. The addition of many of these recent drugs could be possible only with the recognition of the changing character of patents, as applied to medicinal agents, by the governing bodies of the Pharmacopœia and the National Formulary and the coöperative spirit of pharmaceutical industries and other holders of patent rights. It is to be hoped that this spirit of coöperation and realistic approach will continue to be so mutually beneficial to the medical and lay public and to the industry.

With our attention focused upon the possibilities of malaria rampages due to the quinine shortage, we find the introduction of totaquine, which, while originally introduced for the benefit of the Filipinos, will ease the shortage in the homeland for civilian use. In addition, pamaquine naphthoate (plasmoquine) and quinachrine hydrochloride (atebrine) have been standardized to act as auxiliaries to quinine, the former having some advantage over quinine because it destroys the sexual form of the parasite.

Spurred by the knowledge that cocaine is too toxic for hypodermic injection, even though safe for topical application, compounds have been sought which possess low parenteral toxicity. Procaine, of low value topically, meets these requirements in part, but is still not the ideal local anesthetic. The search for this ideal has brought many effective compounds to the attention of the profession and it is reassuring to see three of the most valuable, butacaine sulfate, tetracaine hydrochloride and butyl aminobenzoate now official. Although butacaine has a toxicity parenterally within the cocaine range (thus making it relatively poor for injection), it is rapidly replacing cocaine due to its increased penetrativeness, bringing about a rapid onset of action and long duration coupled with the fact that there is no local ischemia or mydriasis upon local application of this drug to the eye. Tetracaine is rather similar in action to procaine but is effective in smaller concentrations and may be used to advantage in spinal anesthesia. Butyl aminobenzoate is more effective than the ethyl ester as an insoluble anesthetic dusting powder.

Tribromoethanol (avertin), dissolved in amylene hydrate, has been in use for some time for producing general anesthesia by rectum and for supporting general inhalation anesthesia. It has well earned a place in the armamentarium of the surgeon and thus it is advantageous to have official standards. During the past decade, trichloroethylene has become almost a specific in the alleviation of the excrutiating pains of trigeminal neuralgia and suitable standards have been badly needed.

The advent of the sex hormones as structurally known pure chemical entities has marked the past decade as an epoch in the fields of clinical endocrinology and biochemistry. One needs but a glance at the literature in this active field to appreciate the chaos which has marked the situation, a chaos which is in part due to nomenclature. The introduction of estrone and estradiol benzoate may materially aid in clarifying an extremely muddled situation. The fact, however, that these follicular hormones apparently repress pituitary gonadotropic activity brings the hope that these substances may soon be similarly standardized.

It has long been appreciated that there were other chapters to be added to complete the story of the physiology and chemistry of ergot. The isolation and identification of ergotoxine and ergotamine left still a further problem, for it was soon appreciated that these alkaloids could not account for the full activity of the fluidextract upon oral administration. It was the result of the brilliant investigations of Thompson, Dudley and Moir, Stoll and Burckhart and Kharasch and Lagault that a new alkaloid, ergonovine, was discovered and identified and found to account for the major porton of the uterine activity of the drug with none of the so-called sympatholytic activity. The introduction of this new alkaloid and the careful standardization of ergotamine, so useful in the differential diagnosis and treatment

of the migraine syndrome, indicates clearly that the Pharmacopœia is keeping abreast of the times.

Since the last revision period, considerable progress has been made in vitaminology. Many of these findings have led to clinical implications and the result is the standardization of several new products. Menadione, the synthetic vitamin K, is a convenient and acceptable form of the prothrombin factor and bids to find much use in medicine in cases where blood clotting is delayed by improper prothrombin formation. Riboflavin, thiamine hydrochloride and nicotinic acid, all associated with cellular metabolism, were official in the U. S. P. XI by supplement and now nicotinamide may be added to the list. Oleovitamins A and A and D are convenient dosage forms of these well-known vitamins.

Neostigmine, introduced in 1931 for its stimulant action on the intestinal tract, was found by Walker in 1935 to be more effective than physostigmine in the symptomatic treatment of myasthenia gravis. Although much is to be learned about the reaction mechanism of this anti-cholinesterase, this drug in the form of the hydrobromide or methylsulfate appears to give great promise as a stimulant to the parasympathetic nervous system.

While arsphenamine and neoarsphenamine have long proved to be a boon to the syphilitic, nevertheless, other drugs have been badly needed by the syphilologist to supplement these valuable therapeutic agents. These needs may be met, at least in part, by the use of sulfarsphenamine, bismuth and potassium tartrate, bismuth salicylate and mercury salicylate. The introduction of the three sulfa drugs, sulfapyridine, sulfanilamide and sulfathiazole and the introduction of the amoebacide, carbarsone are also very timely and the story of their discovery is so dramatic that anything said here could only detract.

The field of serology has likewise been quite active during the past decade and this activity is reflected in the official recognition given to normal and citrated human plasmas, convalescent sera from measles and scarlet fever patients, human immune globulin and tetanus toxoid.

Surgical supplies such as sutures, gauze, adhesive plasters and the like have now been standardized by the Pharmacopœia. Methods for controlling sterility of those products which are intended to be sterile, for determining the degree of adhesiveness of plasters and the tensile strength of these surgical supplies has long been needed, and the surgeon is grateful for the fact that these products are now subject to precise uniformity. The introduction of products of this type marks a distinct milestone in the progress of medical and pharmaceutical standards.

But the Pharmacopœia is not alone in making such substantial progress. The National Formulary has introduced several drugs and preparations of no little import. Pectin and two pectin pastes show considerable promise as products which will widen in their adaptability. Experiments at the Henry Ford Hospital indicate that pectin will possibly have wide use in the replacement of blood colloids and fluids. The pectin pastes were found by the late Dr. Bernard Fantus to be very helpful carriers for antiseptics, antipruritics and other medicating agents for the treatment of indolent ulcers, bedsores and other skin manifestations coupled with serous exudation. Now that tragacanth and other gums and mucilages are rapidly becoming scarce and since pectin is a native product made from the inner rind of the citrus fruits and from apple pomace, it will undoubtedly act to replace these materials in part.

The introduction of a monograph for phenothiazine, an effective vermicide in veterinary practice, a monograph for merbromin (mercurochrome), which is one of the most widely used household antiseptics, and a monograph for chloroformic solution of coal tar all indicate a spirit of progressiveness.

The careful selection and improvement of the formulas for the diagnostic reagents found in the back portion of the National Formulary represents a valuable contribution to the clinical laboratory and to pharmacists interested in the practices of the clinical laboratory. Adequate and workable directions for preparing solution of this type are often difficult to find and in many cases

almost as many modifications of a basic formula exist as there are workers in the field. The weeding out of the many modifications is consistent with the type of professional service that Pharmacy has always been willing to render.

But progress in the pharmaceutical field as measured by changes in official standards is not to be seen only in terms of the numbers or the quality of new admissions. Many standards for older and well-established drugs have been improved due to progress in the modes of manufacture of these particular products. The limit of color and the introduction of consistency standards for petrolatum and white petrolatum might be cited as examples and the augmenting by the National Formulary of the heavy metals test by the dithizone test for lead in some of the monographs is worthy of more than passing mention.

One of the important scientific contributions which both the National Formulary and the Pharmacopœia have made during the past decade was the introduction of scientific color names into many monographs. These names are in agreement with the ISCC-NBS system of color nomenclature and represent a more precise terminology than that of the past so that a color name now has a uniform and definite connotation to those familiar with the system.

The inclusion in the Pharmacopœia of a test for pyrogens, while probably not representing the last word in such a test, is nevertheless a definite step forward. Also the assay of digitalis by the cat method has many advantages over the frog method in precision and correlation with clinical behavior.

Too much cannot be said for the studies which were made on the storage requirements and on types of containers. The careful definitions of such terms as "well-closed container," etc., is a step forward in this phase of pharmaceutical endeavor. Perhaps of still greater significance is the establishment of methods for determining the light transmission of glass for containers which will be used to hold light-sensitive drugs and pharmaceuticals.

Surely not of least importance is the change of editorial style in both books. This change is most commendable for it will facilitate the use of both books by chemists and other scientifically trained people who may have occasion to take advantage of the methods and the specifications drawn up for these materials.

And now the notches on the Pharmaceutical time candle are to be just half as far apart, as progress in pharmaceutical standardization will be measured in terms of quinquennia rather than decades. Each revision period will be marked by progress—of that we can be sure. But one is forced to wonder if another revision period can ever be so lucrative and so dramatic in the character of the changes which it calls forth.

A Note on *Pinckneya pubens* (Michaux)*

By W. Taylor Sumerford†

This note has been prepared for publication in view of the recent comments in the press as well as in some pharmaceutical circles regarding the possibility of using *P. pubens* as a source of cinchona alkaloids.

Pinckneya (sometimes, *Pinkneya*) *pubens*, Michaux (Fam. *Rubiaceæ*) is described as a shrub or small tree to be found growing in the sandy swamps or coastal plain from West Florida to South Carolina (1).

Because of its habitat, the tree is known as Georgia Bark or Florida Bark. It is also called Bitter Bark for obvious reasons, and Fever Tree, probably because of the use of its bark in treating intermittent

* Received December 26, 1942.

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fevers, or possibly because of the radiant red color of its foliaceous sepals during certain seasons of the year.

According to the 1858 edition of the U. S. Dispensatory (2), a Doctor Law of Georgia used the bark of *P. pubens* to cure 6 out of 7 cases of intermittent fever.

An article published in the *New York Times* calling attention to the need for cultivating cinchona trees in the United States prompted Maisch (3), in 1881, to publish a report on *P. pubens* in which the following information is to be found. "The tree is described in Porcher's 'Resources of the Southern Fields and Forests,' and its bark is said to have some reputation in the treatment of intermittent fevers." Maisch further reported that Farr had detected "cinchonia," one of the lesser alkaloids of cinchona, in the bark of *P. pubens*.

Later Naudain (4) examined an authentic sample of *P. pubens* bark finding no alkaloids, although he did find a crystalline glucoside for which he proposed the name "pinckneyin."

The current edition of the U. S. Dispensatory (5) described the tree, giving its habitat, and states that it is used with alleged advantage in intermittent fevers, but reference is also made to Naudain's negative report (4) in this connection.

This would seem to have settled the question as to whether the cinchona alkaloids exist in *P. pubens*, but the belief persists that the bark of this tree is equal to the bark of cinchona in antimalarial properties and, indeed, provides the cinchona alkaloids. Whether this belief is based on the fact that *P. pubens* and the cinchonas belong to the same botanical family, whether it is based on the bitter taste of *P. pubens* bark or whether it is based on old therapeutic claims is difficult to say.

EXPERIMENTAL

Samples of the bark, leaf and wood of *P. pubens* (identified by Dr. Wilbur Duncan of the Botany Department of the University of Georgia) were collected in Wayne County, Ga., during the month of August, 1942. These samples were dried for a period of 3 months at room temperature, then reduced to a fine powder and subjected to an assay procedure for cinchona alkaloids. Five grams of each sample was treated according to the procedure for determining alkaloids in cinchona bark as detailed in the U. S. Pharmacopœia XI (6). The ether-chloroform extracts of the 3 samples gave a very small residue on evaporation; the amount being the largest with the bark, intermediate with the leaf, and least with the wood. These residues were taken up separately in 25-cc. portions of 5% sulfuric acid. Each portion was filtered, then divided into 5 equal parts, and each part subjected to one specific test for cinchona alkaloids, and to 4 tests for alkaloids in general. Results of these tests are tabulated below.

TABLE I

Reagent	Bark	Leaf	Wood
Bromine T. S. + NH_4OH	Neg.	Neg.	Neg.
HgI_2-2KI T. S.	Neg.	Neg.	Neg.
Picric Acid T. S.	Neg.	Neg.	Neg.
Iodine T. S.	Pos.	Pos.	Pos.
NH_4OH (Excess)	Neg.	Neg.	Neg.

DISCUSSION

These results strongly indicate the absence of cinchona alkaloids, or of any alkaloids, in the bark, leaf or wood of *P. pubens*. It should be recorded, however, that when ammonia T. S. is added in excess to the acid solution in the assay procedure, a strong fluorescence, typical of the cinchona alkaloids, is observed, especially in the case of the bark. It is probable that this reaction led Farr (4) to believe that cinchonine existed in the bark of *P. pubens*.

SUMMARY

The bark, leaf and wood of *Pinckneya pubens* (Michaux), when subjected to the U. S. Pharmacopœial XI procedure for determining alkaloids in cinchona bark and the extracts tested with alkaloidal reagents, gave negative tests for the cinchona alkaloids and for alkaloids in general.

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A Comparison of Two Methods for Evaluating the Relative Efficacy of Disguising Agents for Distasteful Drugs*

By W. Arthur Purdum†

Employing 13 frequently used official syrups and elixirs, Wright (1) has determined their relative palatability as vehicles and relative efficacy in disguising the salty taste of ammonium chloride and the bitter taste of quinine bisulfate. Using the same 13 vehicles, this writer (2) has determined by the threshold method their efficacy in disguising the tastes of ammonium chloride and quinine hydrochloride.

Wright prepared 8% solutions of ammonium chloride and 0.1% solutions of the quinine salt in the respective vehicles and made his determinations on approximately 600 persons as follows. The preparations were given to the individuals in most cases in groups of 4 flavors at one time. The subjects were permitted to retaste the solutions as necessary to decide the order of preference. The results were recorded on the basis of 3 points for first choice, 2 for second, 1 for third and no credit for fourth choice. These points were then expressed as a percentage of a perfect first choice.

The technique of the threshold method, which has been described in detail elsewhere (2), follows in brief. Determine the threshold of taste for a drug in aqueous solution and then make a similar determination in a solution of the vehicle. The difference between these threshold concentrations when expressed numerically is called the "disguising potential" of the vehicle for the drug used. In general for a series of vehicles, the higher the mean disquising potential for a group of subjects, the better the vehicle as a disguising agent for the drug employed.

In order to facilitate a comparison between the results obtained by the threshold method and by the method of Wright, the 2 sets of results for the ammonium chloride determinations appear in Table I. In this table, as well as in Table II, the vehicles are given in the order of decreasing mean disguising potentials. The numerals in the columns captioned "Rank" are given to enable the reader to determine at a glance the relative merit of the vehicles as determined by either method.

TABLE I.—AMMONIUM CHLORIDE DISGUISING EFFICACY OF 13 VEHICLES DETERMINED BY THE THRESHOLD METHOD AND BY WRIGHT'S METHOD

	Threshold Method		Method of Wright	
Vehicle	Rank	Mean Disguising potential*	Rank	Score, %
Syrup of Glycyrrhiza	1	2.16	13	11
Syrup of Raspberry	2	1.81	9	40
Syrup of Citric Acid	3	1.71	5	50
Syrup of Orange	4	1.59	2	61
Aromatic Syrup of Eriodictyon	5	1.55	4	54
Syrup of Cherry	6	1.53	6	47
Syrup of Cocoa, N. F. V	7	1.50	7	42
Syrup of Prepared Cacao, N. F. VI	8	1.47	11	34
Syrup of Wild Cherry	9	1.41	8	41
Syrup of Cinnamon	10	1.38	1	64
Elixir of Glycyrrhiza	11	1.28	10	35
Compound Syrup of Sarsaparilla	12	1.10	3	58
Aromatic Elixir	13	1.03	12	23

* Each figure in this column is the mean for determinations on either 31 or 32 persons.

It is obvious that there is no agreement in the two methods between the results for the syrups of glycyrrhiza, raspberry, cinnamon and compound sarsaparilla. For syrup of prepared cacao, N. F. VI, there is only fair agreement; the threshold method places it in eighth place while Wright's method places it in eleventh. The results for the remaining eight vehicles agree rather closely. The greatest discrepancy occurs in the results for syrup of glycyrrhiza and this might be explained as follows: Wright's subjects voted syrup of glycyrrhiza

* From the laboratory of A. G. DuMez, School of Pharmacy, University of Maryland, Baltimore, Md. This paper is based on part of a thesis presented to the Graduate School, University of Maryland, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Presented to the Scientific Section of the A. PH. A., Denver meeting, 1942.

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the least palatable of the 13 vehicles, the poorest vehicle for the administration of ammonium chloride and also the poorest vehicle in which to administer quinine. This seems to be indicative of general dislike of the glycyrrhiza flavor rather than of poor disguising qualities. Furthermore, many of the persons on whom the threshold determinations were made expressed their dislike of the flavor of glycyrrhiza even though they found it to be one of the best disguises for ammonium chloride. It is believed, therefore, that the threshold method gives an accurate estimate of actual disguising properties without regard for palatability whereas the other method does not. On the other hand, Wright's method is not to be condemned, because the selection of a palatable vehicle is just as important as disguising the taste of the medicament.

TABLE II.—QUININE[a] DISGUISING EFFICACY OF 13 VEHICLES DETERMINED BY THE THRESHOLD METHOD AND BY WRIGHT'S METHOD

	Threshold Method		Method of Wright	
Vehicle	Rank	Mean Disguising Potential[b]	Rank	Score, %
Syrup of Prepared Cacao, N. F. VI	1	4.67	4	67
Syrup of Glycyrrhiza	2	4.41	13	12
Syrup of Cocoa, N. F. V	3	4.40	1	81
Aromatic Syrup of Eriodictyon	4	3.94	3	73
Elixir of Glycyrrhiza	5	3.20	9	27
Compound Syrup of Sarsaparilla	6	3.00	7	39
Syrup of Raspberry	7	2.51	2	77
Syrup of Cherry	8	2.48	5	48
Syrup of Cinnamon	9	2.39	6	41
Syrup of Citric Acid	10	2.33	8	35
Syrup of Wild Cherry	11	2.10	12	14
Syrup of Orange	12	1.74	11	21
Aromatic Elixir	13	1.59	10	27

[a] Wright used quinine bisulfate while quinine hydrochloride was used in the determinations made by the threshold method.

[b] Each figure in this column is the mean for determinations on from 30 to 36 persons.

Results of the determinations by both of the procedures and using quinine salts in the same 13 vehicles are given in Table II.

The most discrepant results in Table II are those for syrup of glycyrrhiza. This was also true when ammonium chloride was used, and the explanation for this has been given above. On the whole, however, the agreement in results is a little better this time than in the experiments in which ammonium chloride was employed.

CONCLUSION

The threshold method evaluates relative taste-disguising efficacy whereas Wright's method appears to evaluate relative palatability and, to a lesser extent, taste-disguising value.

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The Fate of Salol in the Duodenum*

By Alton L. F. Marshall, Jr., and Charles W. Bauer†

The literature contains confusing and contradictory statements about the fate of salol in the intestinal fluids. According to Goodman and Gilman (1), salol is readily hydrolyzed in the intestinal fluids. Scott (2) states that "salol splits up in the alkaline fluids of the intestines." Such statements which occur again and again in the literature are not in agreement with Verzar's (3) work on fat digestion in which he shows that the *p*H of the small intestine is rarely, if ever, on the alkaline side.

Other workers include the effect of pancreatic lipase as an important factor in the decomposition of salol. Sollman (4) states that salol is gradually decomposed in the intestines by the saponifying action of the

* Presented to the Scientific Section of the A. PH. A., Denver Meeting, 1942.

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alkali, pancreatic lipase, and putrefactive bacteria into its components, phenol and salicylic acid. Edmunds and Gunn (5) state that salol is decomposed in the intestine by the fat-splitting ferment of the pancreatic juice. Wood and LaWall (6) are less specific, but say that salol is broken up by the pancreatic juice. Experimental investigation to determine the fate of salol in the duodenal juice, in which there is no agreement, was therefore undertaken.

EXPERIMENTAL

The Effect of Sodium Hydroxide and Bile Salts on the Solubility of Salol.—The solubility of salol in solutions of sodium hydroxide and bile salts in various combinations and concentrations at 38° C. was investigated. Salol is readily saponified in 0.1 *N* sodium hydroxide, but this is much more alkaline than the *p*H of the intestine. It does not undergo saponification in solutions of sodium hydroxide if the *p*H is less than 8.2. Below this *p*H the salol, even after 3 hrs. of contact with the solvent, fails to show saponification and solution in sufficient concentration to obtain a test for phenol with ferric chloride T. S.

These data seem to refute the statements found in the literature that salol is readily saponified by the alkalinity of the intestines.

The Effect of U. S. P. Pancreatin and Bile Salts on the Decomposition of Salol.—The decomposition of salol in solutions of pancreatin, and pancreatin and bile in various concentrations was studied. The mixtures were allowed to digest at 38° C. for 1½ hrs. The decomposition of salol into salicylic acid was determined by titrating the decomposition products with 0.2 *N* sodium hydroxide. There sults show that salol does not decompose. The pancreatin used showed lipase activity by its action upon olive oil. When olive oil and salol were used together, the lipase activity of the pancreatin was materially lessened, suggesting that salol acts as a negative catalyst.

The Effect of Normal Human Duodenal Juice on the Decomposition of Salol.—Since U. S. P. Pancreatin, which is low in lipase, failed to hydrolyze salol, human duodenal juice was used to determine its effect upon salol.

Normal duodenal juice was obtained from the Children's Hospital, Boston, Mass. Two samples were received, 1 from an infant, and 1 from a 6-year-old child. These samples tested at the hospital showed lipase activity. The effect of these duodenal juices on salol was determined with and without the addition of calcium chloride. The solution was then titrated with 0.01 *N* sodium hydroxide, and no decomposition of salol was obtained in either case. This indicates that salol is not decomposed by the pancreatic juice suggested by Sollman (4).

The Fate of Salol-Coated Capsules of Barium Sulfate in the Body.—At the Children's Hospital, Boston, Mass., two boys, one 9 years old and the other 8, were each given 10 salol-coated barium sulfate capsules before breakfast. The 9-year-old boy had normal digestion. The 8-year-old boy had pancreatic fibrosis and was unable to secrete any of the pancreatic enzymes. The progress of the salol-coated barium sulfate capsules was followed by means of an x-ray fluoroscope. The capsules did not leave the stomach for the first 3½ hrs. In the 8-year-old boy, 6 were in the stomach and 4 were in the jejunum at the end of 4 hrs. In the 9-year-old boy, 7 were in the stomach, 2 were in the small intestine and 1 was in the ascending colon in the same period of time. At the end of 6½ hrs. only 2 capsules had disintegrated in each subject, and this disintegration took place well along the intestinal tract. These data seem to indicate that neither normal nor abnormal pancreatic juice causes the saponification of salol in the duodenum of children.

This experiment was repeated by giving 2 salol-coated capsules to an adult. Both capsules left the stomach within 2 hrs., 1 had disintegrated in this period of time. The second capsule disintegrated within 2½ hrs. (Dr. May's induction into the U. S. Army made it impossible to repeat these experiments.) This would seem to indicate that the duodenal juice of a normal adult differs either in composition or degree of activity from that of a normal child. This work will be resumed after the "duration."

The Duration of the Excretion of Salol and Sodium Salicylate in the Urine.—Two grams of salol followed by two tumblerfuls of water was taken and the urine tested at frequent intervals with ferric chloride T. S. At the 2-hr. interval, a positive test for phenol was noted. After 28 hrs., the tests became negative.

This experiment was repeated with 2 Gm. of sodium salicylate. A positive test resulted in 25 min. after ingestion, and became negative after 27½ hrs.

This evidence does not agree with the literature (1) which states that salicylates continue to be eliminated as phenol for a period of several days.

Investigation of the Solubility of Salol and Salicylic Acid in a Solution of Bile Salts.—Verzar (3) has shown in his work on fat digestion that the *p*H of the small intestine is rarely, if ever, on the alkaline side, and that the fats are hydrolyzed to the fatty acids and glycerin. The fatty acid cannot be made soluble by its conversion to the sodium salt unless the *p*H reaches 8 or above. He showed the fatty acid could be absorbed by combining with the bile acids to form a soluble-complex.

Experiments were carried out using both salol and salicylic acid in solutions of sodium taurocholate, sodium glycocholate, and a mixture of the two at 38° C. The salicylic acid dissolved in the solution of sodium taurocholate, but formed a yellow gummy mass in both the sodium glycocholate and the mixture of sodium glycocholate and taurocholate. The

salol did not appear to dissolve in any of these solutions.

Since salol is neither hydrolyzed by normal duodenal juice nor dissolved by bile salts, it is doubtful if the bile salts will react with salol in the manner in which they behave with fatty acids.

Decomposition of Salol by Minced Liver and Minced Kidney.—Our experiments show that salol is not readily broken down in the duodenum, if at all. But the fact that salol appears in the urine as phenol indicates it is either hydrolyzed further along the intestinal tract, or is absorbed as some soluble-complex into the blood stream and then decomposed by some organ on its route to the kidney. We were unable to obtain normal intestinal secretions beyond the duodenum for this work. In order to determine the effect of liver and kidney tissue on salol—if it were absorbed into the blood stream as some soluble-complex—the following experiment was made.

A 2-day-old cat's liver kept in physiological salt solution in a refrigerator was minced, and to this was added salol, buffered with phosphate solution at a *p*H of 7.4 and the mixture was then placed in an oven at 38° C. for 1 hr. The mixture was filtered, and a strongly positive phenol test was obtained with ferric chloride T. S. in the filtrate. This experiment was repeated with 2-day-old cat's kidney, and again a strong positive phenol test with ferric chloride T. S. was noted. The results of these experiments seem to indicate that if salol were absorbed as some soluble-complex, it could be converted into phenol by either the liver or the kidney.

CONCLUSIONS

1. Salol is not readily saponified by the alkalinity of the duodenum.

2. Active pancreatic lipase does not hydrolyze salol.

3. Normal duodenal juice does not decompose salol.

4. Salol is not decomposed in the duodenum of a child having a normal pancreatic secretion nor in the duodenum of a child that has no pancreatic enzymes.

5. Salol will give a positive phenol reaction in the urine within 2 hrs. after ingestion. The urine fails to give a positive test for phenol after 28 hrs.

6. Sodium salicylate appears in the urine within 25 min. and fails to give a positive phenol test in the urine after 27½ hrs. This indicates faster elimination of the salicylates than is ordinarily believed possible.

7. Salol does not appear to dissolve in either bile acids or bile salts.

8. Salol is converted into phenol by either minced liver or minced kidney tissue of the cat.

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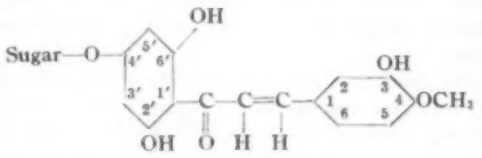
CORRECTION—March, 1943, p. 75, "The Chemical Nature of Vitamin P" by Ralph H. Higby. The formula for "chalcone" should read as follows:



The Alkaloids of *Lunasia amara**

By Frank A. Steldt and K. K. Chen†

Lunasia amara Blanco, Fam. *Rutaceæ* is a shrub indigenous to Northern Luzon, Mindanao and Palawan, Philippine Islands, and grows in thickets and forests at low and medium altitudes (1). The plant can easily be identified by its flowers, leaves and seeds as shown in Fig. 1, or on the basis of even a sterile, leafy branch (2). Botanically, it is synonymous with *Rabelaisia philippinensis*, *Lunasia philippinensis*, *L. reticulata* and *Pilocarpus amara*. It is said that natives of the Philippine Islands employed the plant for making an arrow poison (3).

Earlier workers, Loher, Rosenthal and Gärtner, mentioned by Plugge (4), believed that the active principle was a glycoside having a digitalis-like action. Plugge (4), himself, claimed to have succeeded in isolating the same, and named it rabelaisin. A year later, Lewin (3) obtained a crystalline alkaloid, lunasine, from the bark of *Lunasia amara*. Boorsma (5, 6) reported the presence of 3 alkaloids—lunacrine, m. p. 87–88° C.; lunasine, m. p. 143° C.; and lunacridine, m. p. 82–84° C. This author stated that the specimens giving rise to a glycoside as disclosed by the earlier investigators were confused with the bark of *Lophopetalum toxicum*—a very reasonable suspicion, because subsequent studies all resulted in alkaloids. Thus, Brill and Wells (7) reported the presence of lunasine, Wirth (8) found 2 alkaloids, and Dieterle and Beyl (9) succeeded in isolating 3 alkaloids—lunacrine, m. p. 95.5° C.; lunasine, m. p. 188° C.; and lunacridine, m. p. 231° C.

The pharmacological action of the *Lunasia* alkaloids has been elucidated by Lewin (3), Boorsma (6) and Wirth (8). Boorsma observed that lunacrine, lunacridine and lunasine all caused systolic standstill of the frog's heart.

In the systematic investigations of digitalis-like plants, it was our desire to settle once and for all whether or not the constituents of *L. amara* (*Rabelaisia philippinensis* of Plugge) were alkaloids, and whether or not they had a digitalis-like effect—comparable to the *Erythrophloeum* alkaloids. Since the bark could only be collected in remote provinces, mistakes in identification were very easy, especially by laymen. For example, our first specimen, upon chemical examination, gave rise to β-amyrin and a water-soluble substance containing no nitrogen. It was through the untiring efforts of Messrs. A. H. Brown and T. H. Eckerson, of our own organization, and the close coöperation of Dr. Eduardo Quisumbing, Bureau of Science, Philippine Islands, that a quantity of authentic material was finally obtained. The drawings in Fig. 1 vouch for this fact.

Fig. 1.—*Lunasia amara* Showing Flowers, Fruits, Pods and Seeds.

* From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

Presented to the Scientific Section of the A. PH. A., Denver meeting, 1942.

† The authors are indebted to Messrs. Robert C. Anderson, Clarence E. Powell, E. Brown Robbins and Harold M. Worth.

Briefly, 4 alkaloids were isolated in crystalline form by the common immiscible solvents—lunacrine, $C_{16}H_{19}O_3N$; lunacridine, $C_{17}H_{23}O_4N$; lunamarine, $C_{18}H_{15}O_4N$; and lunamaridine, $C_{16}H_{15}O_2N$. There was no evidence of lunasine, which might be attributed to our use of sodium hydroxide since Boorsma (5, 6) stated that this alkali decomposed it to lunacridine. The melting point and color reactions of our lunacridine closely agree with those of Boorsma's alkaloid (5, 6) bearing the same name. Our lunamarine is probably identical with Dieterle and Beyl's lunacridine (9), for the melting points and chemical composition of both products are comparable. The new name is adopted in order to eliminate confusion. Pharmacologically, none of the alkaloids showed any digitalis-like action. Lunacrine, lunacridine and lunamarine reduced arterial blood pressure in cats. Lunacrine and lunacridine inhibited isolated rabbits' intestines, while lunamarine stimulated both isolated rabbits' uteri and intestines.

CHEMICAL PART

A quantity of 8 Kg. of air-dried bark of *Lunasia amara* was pulverized and percolated with ether. The extract was evaporated to dryness under diminished pressure. The residue, upon treatment with ethyl acetate, acetone and alcohol successively, gave rise to 2 waxy, amorphous substances melting at about 200–203° C. and 135–137° C., respectively. Their amounts were too small to allow further purification and characterization.

The powdered bark was then subjected to alcohol percolation until exhaustion. After the percolate was evaporated under vacuum distillation to a syrupy consistency, it was treated several times with a hot 10% solution of hydrochloric acid. The acid mixture was decanted, made alkaline with a 10% solution of sodium hydroxide, and repeatedly extracted with ether. The alkaline solution was further extracted with chloroform for the removal of remaining alkaloids.

The ether extract was retreated with hydrochloric acid for the sake of purification. The alkaloids were regenerated by sodium hydroxide, and reëxtracted with ether. The latter was evaporated to dryness, and the residue was extracted with hot water containing 5% alcohol. On standing, long silky needles appeared. This was lunacrine. The residue was further treated with hot benzene, and an additional amount of impure lunacrine was obtained by evaporation to dryness. Purification was finally effected by dissolving the mass, chiefly lunacrine, in hydrochloric acid. When the mixture was again evaporated to dryness, lunacrine hydrochloride was readily removed by hot acetone, and crystallized from it on standing.

The acetone-insoluble portion, very small in quantity, gave rise to a new alkaloid, which we named lunamaridine. The latter was finally crystallized from alcohol by the addition of a few drops of water. Apparently, this alkaloid is very weakly basic, for it did not form the hydrochloride in the presence of hydrochloric acid.

The remainder of the ether extract, very sparingly soluble in either water with 5% alcohol or benzene, was dissolved in a methanol-water mixture. Upon standing, an alkaloid, different from lunacrine and lunamaridine, crystallized out. This is provisionally called lunamarine since its properties and composition suggest that it is not the same as Boorsma's lunacridine (5, 6).

The chloroform extract from the original alkaline mixture was shaken with hydrochloric acid, 10% solution, and reëxtracted with chloroform upon the addition of sodium hydroxide, also 10% solution. This was repeated several times. When the final chloroform solution was treated with ether, a precipitate was formed and separated by filtration. In spite of repeated attempts, no crystalline products could be obtained. A chromatographic experiment was finally resorted to. The whole was dissolved in a mixture of ethyl acetate and chloroform, 1:1, and eluted through a column of aluminum oxide with the same mixture of solvents. The last fraction contained a residue which crystallized from ethyl acetate, and proved to be lunamarine.

The chloroform mother liquor, after precipitation with ether, was again subjected to extraction with immiscible solvents by acidification and alkalinization, alternately. The last chloroform extract was diluted with ether and allowed to evaporate spontaneously at room temperature. Crops of crystals, formed on the surface and recrystallized from ethyl acetate, were identified to be those of lunacridine.

Lunacrine crystallizes from water containing 5% alcohol in colorless fine needles, and melts at 95.5° C. When dried gradually at 110° C., it melts at 115° C. It has a specific rotation of $[\alpha]_D^{28} -58°$ in 95% alcohol. It is soluble in alcohol, ether, hot water, ethyl acetate and benzene. Solutions of lunacrine exhibit a blue fluorescence in ultraviolet light. The total yield of this alkaloid in pure form was 3 Gm. Like most alkaloids, it precipitates Mayer's reagent. When lunacrine is dissolved in concentrated sulfuric acid with a few crystals of potassium dichromate, a violet color appears, quickly turning to reddish brown. Elementary analysis yields results compatible with the empirical formula $C_{16}H_{19}O_3N$ (calculated, C 70.29, H 7.01, N 5.13; found, C 70.37, 70.30, H 7.16, 7.15, N 5.23, 5.45). It differs from Wirth's formula (8) by having one less H-atom.

Lunacrine easily forms a hydrochloride, which crystallizes from acetone in fine needles, and melts at 164–165° C. with effervescence. Its specific rota-

tion is $[\alpha]_D^{28}$ −23° in water. Analytical data agree with the formula $C_{18}H_{19}O_2N.HCl$ (calculated, Cl 11.45; found, Cl 11.19, 11.23).

Lunamaridine, as crystallized from alcohol with a few drops of water, is in small needles, and melts at 209–210° C. The outcome of analysis suggests the formula $C_{18}H_{15}O_2N$ (calculated, C 75.85, H 5.97, N 5.53; found, C 75.30, 75.18, H 5.73, 5.64, N 5.31, 5.36). Heretofore this alkaloid has not been described.

Lunamarine crystallizes in colorless, stout prisms, melts at 245–246° C., and is optically inactive. It is soluble in methanol, ethanol, ether and chloroform, but very slightly soluble in benzene and water. The alcoholic solution of lunamarine shows a brilliant blue fluorescence in ultraviolet light. The total yield of the pure alkaloid was 2 Gm. It precipitates Mayer's reagent, and colors concentrated sulfuric acid yellowish brown in presence of potassium dichromate. Analytical results are in agreement with the formula $C_{18}H_{11}O_4N$ (calculated, C 69.87, H 4.89, N 4.53; found, C 69.86, 70.05, H 4.70, 4.77, N 4.57, 4.60). In all probability our lunamarine is identical with Dieterle and Beyl's alkaloid (9) which they named lunacridine.

Lunacridine crystallizes from ethyl acetate in large, stout, colorless needles, softens at 79° C., and melts at 83.5° C. It has a specific rotation of $[\alpha]_D^{28}$ + 31.6°. It is soluble in methanol, ethanol, ethyl acetate, chloroform and ether, but very sparingly soluble in water. Its solutions display a weak blue fluorescence in ultraviolet light. When dissolved in concentrated sulfuric acid, lunacridine shows a bluish green color on the addition of potassium dichromate. The total yield of the crystalline alkaloid was approximately 4 Gm. Based on elementary analysis, an empirical formula of $C_{17}H_{21}O_4N$ is proposed (calculated, C 66.85, H, 7.59, N 4.59; found, C 66.56, 66.32, H 7.13, 7.40, N 4.58, 4.54) The low melting point and other properties of this alkaloid agree well with those of Boorsma's lunacridine (5, 6)—and thus the retention of the same name.

PHARMACOLOGICAL PART

Lunacrine and especially its hydrochloride cause a sharp burning sensation on the tongue, but lunacridine and lunamarine are practically tasteless.

When injected into the ventral lymph sac of frogs, none of the 4 alkaloids produced a systolic standstill of the ventricle at the end of an hour. With the exception of lunacrine hydrochloride, which is soluble in water to the extent of 1–2%, the other 3 alkaloids required alcohol for complete solution—a 0.1% solution of lunamarine and a 0.2% solution of lunacridine each needed 28.5% alcohol; and a 0.06% solution of lunamaridine, 19% alcohol, all by volume. The doses employed varied—lunacrine, 0.6–1.6 mg./Gm.; lunacridine, 0.4–0.98; lunamarine, 0.12–1.7; and lunamaridine, 0.04–0.08. It can be stated, therefore, that *Lunasia amara* and its alkaloids do not belong, pharmacologically, to the digitalis group.

In etherized cats, intravenous injection of lunacrine hydrochloride in the dose of 15 mg., or lunacridine in the dose of 10 mg., resulted in a moderate fall of blood pressure with recovery in about 2 min. The amplitude of respiration diminished during the fall of pressure. Similarly, lunamarine induced a primary drop of carotid pressure followed by a secondary rise, as shown in Fig. 2.

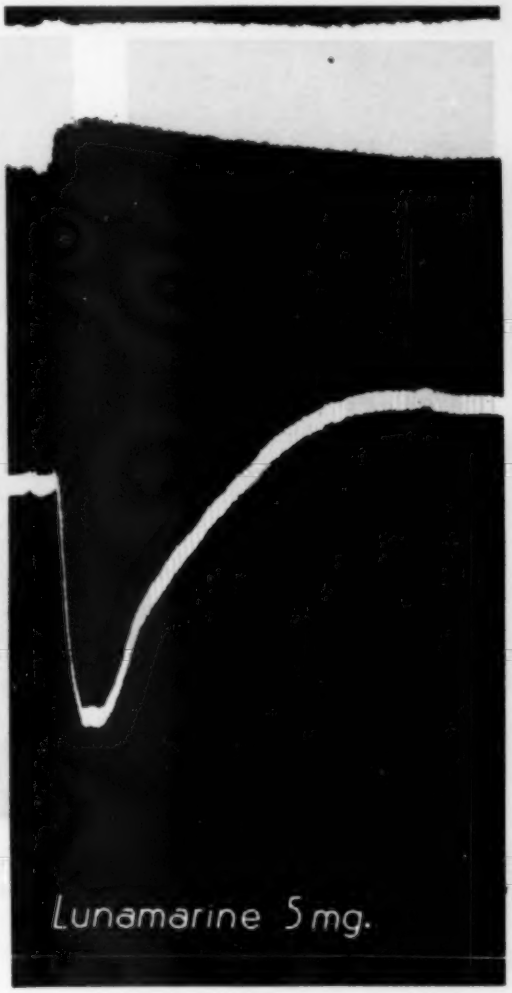


Fig. 2.—Action of Lunamarine on Blood Pressure.
Cat, male, weighing 1.956 Kg., received 5 mg. of lunamarine by the femoral vein. Note the fall of blood pressure and the secondary rise.

The action of lunacrine, lunacridine and lunamarine on isolated rabbits' uteri and intestines was studied in several experiments. The results were uniform. In the concentration of 1:100,000, lunacrine and lunacridine slightly inhibited the peristaltic movements of rabbits' intestines, but had practically no effect on the uteri. Lunamarine, on the other hand, stimulated both intestines and uteri in the concentration of 1:200,000. An example is shown in Fig. 3.

Solutions of lunacrine, lunacridine and lunamarine were injected intravenously into rabbits, and their blood sugar was determined hourly for a period of 6 hrs. by the Hagedorn-Jensen method (10). The doses used were: lunacrine, 50 mg./Kg.; lunacridine 5 mg./Kg.; and lunamarine, 0.4 mg./Kg. No significant changes in blood sugar were observed.

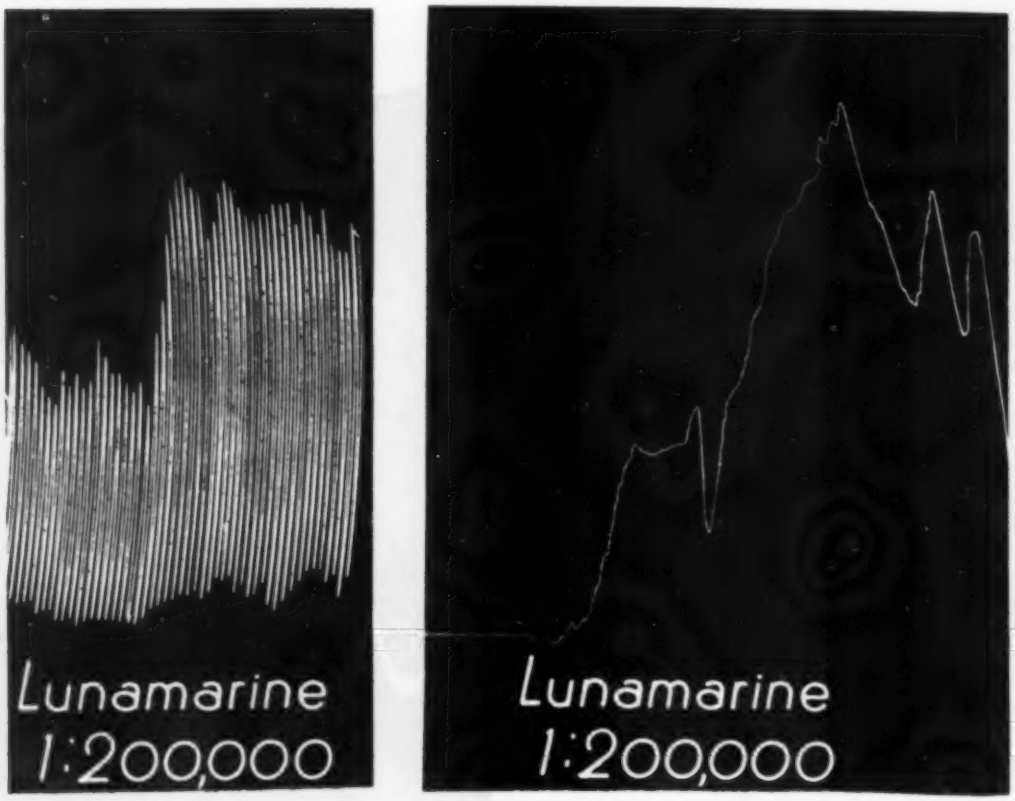


Fig. 3.—Action of Lunamarine on Isolated Rabbit's Intestines and the Uterus. The stimulating effect is obvious in both experiments.

The acute toxicity of 3 alkaloids was investigated. The median lethal dose of lunacrine hydrochloride with its standard error by intravenous injection in albino mice was found to be 78.7 ± 3.8 mg./Kg.—33 animals being employed. The median lethal dose of lunacridine by mouth was determined in a group of 40 mice to be 1097 ± 167 mg./Kg. With lethal doses of both alkaloids, convulsions followed by prostration and respiratory failure occurred before death. Lunamarine was given orally to a group of 12 mice. Doses as high as 1000 mg./Kg. were not fatal.

SUMMARY

1. Four alkaloids have been isolated from the bark of *Lunasia amara:* lunacrine, $C_{16}H_{19}O_3N$; lunacridine, $C_{17}H_{23}O_4N$; lunamarine, $C_{18}H_{15}O_4N$; and lunamaridine, $C_{16}H_{16}O_2N$.

2. None of the 4 alkaloids studied has a digitalis-like action on the frog's heart.

3. Lunacrine, lunacridine and lunamarine cause a fall of blood pressure in etherized cats.

4. Lunamarine stimulates the isolated rabbit's uterus and intestines, while lunacrine and lunacridine inhibit slightly the peristaltic movements of isolated intestines.

5. The acute toxicity of lunacrine hydrochloride and lunacridine has been determined in white mice by intravenous injection and by mouth, respectively.

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Petro-Waxes in Ointment Bases*

By Clark Ice† and Loyd E. Harris‡

The United States Pharmocopœia, Eleventh Revision, has the following statement under general notices: "In the official ointments which contain yellow or white wax or paraffin as stiffening agents, the proportions of these and the other fatty substances directed in the official formulas may be varied to maintain a suitable consistence under different climatic conditions. . . ." Apparently the paraffin has no other function than to stiffen or raise the melting point of the ointment. Beeswax, both bleached and unbleached, has been shown to have the additional property of increasing the "water number" or "water value" of the ointment to a limited extent (1). It is believed that the absorption of medicaments from ointments is increased, in some instances, by the presence of water in the finished preparaton. Where the percentage of water is comparatively large, an added advantage is gained by making the treated areas more readily washed or cleaned.

Certain other waxes have been found to have properties similar to beeswax (2). One of these is ceresin, which is a mixture of hydrocarbons produced by purification of ozokerite, which is found occurring naturally in the area of Boryslaw in Galicia (3). This substance has the ability to stiffen ointment bases and at the same time it serves to minimize or eliminate "oil leakage" or separation of oils from the ointment when water is incorporated. Some consideration has been given to the possibility of incorporating ceresin into the formula of a standard ointment base in this country. Such a proposed formula included ceresin, mineral oil, cetyl alcohol, stearyl alcohol and sodium lauryl sulfate. Ceresin has been included in other pharmacopœias ("Deutsche Arzneibuch," Fifth edition, 1910) to give formulas that would be miscible with an abundance of water (4).

The Bareco Oil Company[1] of Barnsdall, Okla., prepares a series of "Petro-waxes," one of which melts at about 160° F. It is a white microcrystalline wax that is available in quantity. It was thought that due to the microcrystalline structure of this wax, it might make stable ointments containing as much as 50% water, just as effectively as ceresin. It was known that the petro-waxes were being used in the preparation of cosmetics, especially cold creams.

The separation of the petro-waxes from petroleum oils was originally done by a sweating process. However, in recent years, extraction by solvents has found wide appli-

* From a thesis submitted by Clark Ice to the Faculty of the Graduate School of the University of Oklahoma, Norman, Okla., in partial fulfillment of the requirements for the degree of Master of Science.

Presented before the Sub-section of Pharmacy, American Association for the Advancement of Science, December 30, 1941, in Dallas, Texas.

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[1] The authors wish to express their appreciation to the Bareco Oil Company for furnishing the Bareco Wax used in this investigation and for the making and printing of the photomicrographs. The photomicrographs referred to in this paper are to be found in the original dissertation in the library of the University of Oklahoma.

cation. A large number of solvents have been suggested and many of them have been found useful. Liquid propane, pyridine, oxidized wax, ethanesulfonyl chloride with benzene, ethyl acetate with benzene, acetone and tetrahydronaphthalene, glycerol tristearate, carbon tetrachloride, and ethlene dichloride have been found satisfactory.

The nature of wax crystals, as well as waxes mixed with various other substances, has been studied. In our investigation, photomicrographs were made of several blends of the 160° F. wax obtained by mixing several ointment bases in varying percentages at temperatures slightly above the melting points and allowing to congeal, while stirring. Photomicrographs of some of the pure substances were also taken. A comparison of the photographs may give some information as to the effects on the crystalline structure of some ointment bases by the addition of various waxes. There may also be a correlation of the crystalline structure changes with the effect on the melting points of the bases and on the oil retention properties, as well as possibly certain other properties.

EXPERIMENTAL

Melting Point Elevation.—Since the melting point of any ointment base is an essential characteristic, it was important to determine the melting point of several common bases on the addition of increasing percentages of the stiffening agents under examination. This shows whether or not the agent is capable of giving bases of the desired melting point, within the allowable amount of the agent which may be added. This means, as a general rule, that there must be a sharp increase in the melting point in the lower percentages of the agents added for this purpose.

Blends were prepared by heating the components together on a water bath until melted, then stirring until solidification took place. Melting points were determined by the capillary method. Open capillaries were used. They were filled with equal amounts, immersed in the water bath to a uniform depth and heated at the same rate for all determinations. The temperature at which the material began to flow, under the hydrostatic pressure, was taken as the melting point.

TABLE I.—MELTING POINTS OF WHITE PETROLATUM BLENDED WITH VARYING PERCENTAGES OF BARECO 160 WAX

Sample No.	Per Cent Wax	Per Cent Petrolatum	Melting Point*
..	0	100	110
8	1	99	112
9	2	98	116
10	3	97	119
11	4	96	121
12	5	95	122
13	10	90	134
14	15	85	140
15	20	80	144
16	25	75	148
17	30	70	150
18	35	65	152
19	40	60	154
..	100	0	168

*All melting points given in this and the following footnote tables are in degrees Fahrenheit.

TABLE II.—MELTING POINTS OF ANHYDROUS LANOLIN BLENDED WITH VARYING PERCENTAGES OF BARECO 160 WAX

Sample No.	Per Cent Wax	Per Cent Anhydrous Lanolin	Melting Point
..	0	100	106
27	1	99	108
28	2	98	110
29	3	97	112
30	4	96	120
31	5	95	126
32	10	90	146
33	20	80	156
..	100	0	168

TABLE III.—MELTING POINTS OF LARD BLENDED WITH VARYING PERCENTAGES OF BARECO 160 WAX

Sample No.	Per Cent Wax	Per Cent Lard	Melting Point
..	0	100	92
34	1	99	94
35	3	97	98
36	5	95	120
37	10	90	134
38	20	80	154
..	100	0	168

TABLE IV.—MELTING POINTS OF BASE "A" WITH VARYING PERCENTAGES OF BARECO 160 WAX

Sample No.	Per Cent Wax	Per Cent of "A"	Melting Point
..	0	100	112
44	3	97	120
45	5	95	122
46	10	90	126
47	20	80	140
..	100	0	168

Tables I to VIII show the results of the addition of various waxes to white petrolatum, lard, anhydrous lanolin and to 3 blended bases. Base "A" consisted of white petrolatum 95 and anhydrous lanolin; base "B" was a mixture of lard 95 and anhydrous lanolin 5; base "C" was a mixture

of white petrolatum 53.3, lard 41.8 and anhydrous lanolin 4.9. All of the materials used were of U. S. P. quality with the exception of the petro-wax, the specifications of which are given below.

The Bareco 160 petro-wax used in this investigation had the following properties:

Specific gravity at 60° F.	0.92–0.94
Specific gravity, at 212° F.	0.80–0.82
Melting point (A. S. T. M. D-127–30)	160–165
Saybolt universal viscosity, 212° F.	75–100
Flash, C. O. C.	Above 500° F.
Color	White
Iodine number	Less than 1
Saponification number	Less than 2
Acid number, maximum	0.1
Ash	Trace
Odor	None
Taste	None
Penetration at 77° F.	15/25

TABLE V.—MELTING POINTS OF BASE "B" WITH VARYING PERCENTAGES OF BARECO 160 WAX

Sample No.	Per Cent Wax	Per Cent "B"	Melting Point
..	0	100	90
48	3	97	134
49	5	95	138
50	10	90	144
51	20	80	152
..	100	0	168

TABLE VI.—MELTING POINTS OF BASE "C" WITH VARYING PERCENTAGES OF BARECO 160 WAX

Sample No.	Per Cent Wax	Per Cent "C"	Melting Point
..	0	100	100
52	3	97	110
53	5	95	116
54	10	90	128
55	20	80	144
..	100	0	168

Following this series of investigations, it was decided to determine the effects of another "Petro-wax" that is made by the Gulf Refining Company and designated as Gulf Petrowax "A," on the melting point of the white petrolatum. The wax had the following properties:

Melting point (A. S. T. M. D-127–30)	160.5
Saybolt universal viscosity, 210° F.	70.6
Neutralization number	0.01
Penetration, A. S. T. M. D 5–25, at 77° F., 200 G. 5 sec.	44
Color	Medium amber

The data are given in Table IX.

TABLE VII.—MELTING POINTS OF WHITE PETROLATUM WITH VARYING PERCENTAGES OF WHITE BEESWAX

Sample No.	Per Cent White Wax	Per Cent Petrolatum	Melting Point
87	3	97	110
88	5	95	112
89	10	90	115
90	20	80	119
..	100	0	124

TABLE VIII.—MELTING POINTS OF WHITE PETROLATUM WITH VARYING PERCENTAGES OF YELLOW BEESWAX

Sample No.	Per Cent Yellow Wax	Per Cent Petrolatum	Melting Point
91	3	97	112
92	5	95	114
93	10	90	122
94	20	80	129
..	100	0	142

TABLE IX.—MELTING POINTS OF WHITE PETROLATUM WITH VARYING PERCENTAGES OF GULF PETROWAX "A"

Sample No.	Per Cent of Gulf Wax	Per Cent Petrolatum	Melting Point
..	0	100	108
95	3	97	112
96	6	94	118
97	10	90	123
98	20	80	128
99	30	70	131
..	100	0	154

VISCOSITY DETERMINATIONS

The viscosity of ointment bases has not been given much consideration. In order to obtain some information, 3 blends were prepared. The components were heated together on a water bath until melted, then stirred until cool. Blend "D" was made up of white petrolatum 95 and Bareco 160 Wax 5. Blend "E" was made with 90 parts white petrolatum and 10 parts of Bareco 160 Wax. Blend "F" contained 75 parts of white petrolatum with 25 parts of white beeswax. Pure white petrolatum was also used.

The Saybolt universal viscosities were then determined for each blend at different temperatures. The results are given in Tables X, XI, XII and XIII.

TABLE X.—SAYBOLT UNIVERSAL VISCOSITY OF WHITE PETROLATUM

Temp., ° F.	Viscosity, Sec.
122	157
134	137
140	119
144	113

From the data obtained from the melting-point determinations and from the viscosity measurements, it was believed that the Bareco 160 Wax could be used to replace beeswax in U. S. P. formulas. Accordingly, a series of U. S. P. ointments were prepared according to the official formula. A second series were prepared using Bareco 160 Wax in place of the beeswax. One hundred grams of the official formula was prepared in each of the following ointments; Simple ointment, Tannic Acid, Phenol, and Zinc Oxide. The amounts of the Bareco 160 Wax used were respectively, 2.5, 2.5, 3 and 2. Each of the ointments so prepared compared favorably in every detail with the standard preparations.

TABLE XI.—SAYBOLT UNIVERSAL VISCOSITY OF BLEND "D"

Temp., ° F.	Viscosity, Sec.
122	360
134	185
140	136
144	130

TABLE XII.—SAYBOLT UNIVERSAL VISCOSITY OF BLEND "E"

Temp., ° F.	Viscosity, Sec.
122	485
140	174
144	166

TABLE XIII.—SAYBOLT UNIVERSAL VISCOSITY OF BLEND "F"

Temp., ° F.	Viscosity, Sec.
116	488
128	127
134	99
138	96

A Water-Absorptive Ointment Base.—A sample of a water-absorptive base was prepared according to the formula:

Sodium Lauryl Sulfate	2.0
Cetyl Alcohol	12.0
Stearyl Alcohol	6.0
Ceresin (White)	5.0
Liquid Petrolatum	45.0

A sample of this base had been found to be stable, not only in this form, but when as much as 50% of water had been incorporated.

It is known that many bases which appear to be stable to "oil leakage," will lose oil if their crystalline structure is broken. This is usually done by punching the forefinger down into the center of the sample. Oil may then be seen to separate and accumulate in the well. When treated in this manner, the above sample was found to separate an appreciable amount of oil over a period of 2 months, standing at room temperature.

The formula was now modified by replacing the ceresin with 3.2 Gm. of Bareco 160 Wax. The oil separation was noticeably less marked than in the original. The last-mentioned formula was also found to form stable emulsions with the incorporation of water, up to as much as 50%; higher percentages were not tried.

SUMMARY

1. Melting points have been determined of ointment bases made by blending varying percentages of Bareco 160 Wax with white petrolatum, anhydrous lanolin, lard and certain mixtures of them.

2. Certain U. S. P. ointments were prepared by substituting Bareco 160 Wax in calculated amounts, for beeswax.

3. Bareco 160 Wax was used to replace ceresin in the preparation of an ointment base and it was found to be less subject to "oil leakage" than when ceresin was used. The formula with the Bareco 160 Wax was also found capable of forming stable ointments when as much as 50% of water is incorporated.

CONCLUSION

Bareco 160 Wax, one of a series of "Petrowaxes" prepared by the Bareco Oil Company, of Barnsdall, Okla., forms a mixture of suitable consistency and smoothness, with the common ointment bases. It raises the melting point satisfactorily, giving a satisfactory stiffening effect, and gives a suitable viscosity to the sample.

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Derivatives from Hydrogenated Castor Oil

II. Glycol Esters of 12-Hydroxystearic Acid*

By Saul A. Bell† and Abraham Taub‡

Within recent years synthetic fatty acid esters have been replacing, in considerable degree, the natural fats, oils and waxes in cosmetics and pharmaceuticals. The use of these synthetic materials has resulted in more consistent qualities of finished products and has made possible new and different preparations with more useful characteristics not obtainable with the natural fats and waxes.

In a previous paper (1) the isolation of 12-hydroxystearic acid from a commercial hydrogenated castor oil and the synthesis of a series of 12 normal primary alkyl esters of the acid were described, and some of their properties and applications in ointments and creams were indicated. The present paper deals with the esters of this acid with the dihydric alcohols—ethylene glycol, propylene glycol (propandiol-1,2) and trimethylene glycol (propandiol-1,3). Besides the monoesters of these 3 glycols, small amounts of the diesters of ethylene glycol and trimethylene glycol were also prepared. Preliminary studies were made of the properties and characteristics of these pure compounds and some compositions containing them.

The 12-hydroxystearic acid employed in these syntheses was isolated as previously described (1), in good yield and practical purity from a commercial hydrogenated castor oil having an iodine value less than 2 and melting at about 86° C. The esters obtained were likewise in good yield and high purity.

EXPERIMENTAL

Preparation of the Glycol Esters of 12-Hydroxystearic Acid.—In the esterification of 12-hydroxystearic acid with the glycols, it was of primary importance to control the extent of esterification; that is, to effect the formation of either monoester alone or diester alone. Simple combination of glycol and fatty acid by long heating at high temperatures as ordinarily carried out with the saturated higher fatty acids (*e. g.*, palmitic and stearic acids) results invariably in mixtures which must be resolved by fractional crystallization (2, 3).

Practically pure glycol monoesters of some of the higher fatty acids can be prepared, according to Hilditch and Rigg (4) by carrying out the esterification in phenol with a ten-molar excess of glycol at comparatively low temperatures in the presence of an efficient aryl sulfonic acid catalyst and using a stream of dry CO_2 to remove water as formed.

This method and similar ones were found to be unsuitable for esterification of 12-hydroxystearic acid, because this hydroxy acid was dehydrated rather rapidly at the temperatures required for satisfactory yields.

The method that was finally developed for the preparation of the glycol esters consisted of refluxing the hydroxy acid together with an excess of glycol and a small amount of acid catalyst in a water-immiscible organic solvent, using a distillation trap to collect the water carried over mechanically by the latter, until sufficient aqueous layer had separated in the trap to indicate that the desired degree of esterification had been reached. It was necessary in the preliminary experiments to take the refractive index of this aqueous layer in order to estimate the amount of water present since in some cases considerable quantities of glycol were also carried over. After the optimum conditions of time and temperature were determined for these esterifications, this measurement served as an indicator of the completeness of the reaction desired.

Preliminary experiments were carried out in toluene using equal weights of acid and solvent, 2 moles of glycol per mole of acid, and an amount of catalyst, toluene *p*-sulfonic acid, equal to 5% of the weight of the acid. The reaction mixture was refluxed at 135–145° C. until approximately the theoretical amount of water was obtained.

The solvent was removed from the reaction mixture under reduced pressure, the residue taken up in ether and washed thoroughly with a dilute aqueous solution of potassium carbonate. The ether solution was dried with anhydrous sodium sulfate and successive crops of material collected by concentra-

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* Abstracted in part from the dissertation submitted by Dr. Bell to the Faculty of the College of Pharmacy, Columbia University, New York, N. Y., in partial fulfillment of the requirements for the degree of Doctor of Pharmacy.

Presented to the Scientific Section of the A. PH. A., Denver meeting, 1942.

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TABLE I.—ESTERIFICATION OF 50 GM. 12-HO-STEARIC ACID WITH ETHYLENE GLYCOL (0.5% $MePhSO_3H$)

Solvent, Gm.		Moles Glycol per Mole Acid	Time, Min.	Temp., ° C.	H_2O[a] Collected, Cc.	% Glycol[b] in H_2O	Yield, Gm.	M. P., ° C.	Iodine No.	Hydroxyl No.[c]
Toluene	50	2	30	135–140	3.19	26	52.6	....[d]	0.44	291.8
	50	10	40	135–140	3.60	47	39.1	65.8–8.3	0.40	317.1
Xylene	50	2	40	145–150	3.13	7	55.1	....[d]	0.79	280.7
	50	10	30	160	3.45	55	49.8	67–8.6	0.33	311.2
	50	10	80	160–165	5.10	22	54.5[e]	60–6	1.02	303.2
	50	10	270	150–170	4.83[f]	..	57.1	....[d]	4.20	276.8
	100	10	120	155–160	4.95	25	55.1	61–5	0.79	308.0

[a] Represents volume of aqueous layer separated in moisture determination trap.
[b] Approximated from refractive index of aqueous layer.
[c] Hydroxyl numbers were determined by the F. A. C. method. (*Cf. Ind. Eng. Chem., Anal. Ed.*, 12 (1940), 382.) Theoretical for ethylene glycol mono-12-hydroxystearate is 325.9, and for the diester, 179.1.
[d] No melting points were taken because of the noncrystalline wax-like character of these products.
[e] Some loss here.
[f] Represents weight of water as calculated from weight of aqueous layer obtained corrected for per cent glycol it contained as approximated from refractive index.

tion of the solution. These crops were recrystallized and combined into fractions of similar melting point. Determination of hydroxyl values of these fractions indicated that the products of esterification with ethylene glycol and trimethylene glycol were mixtures of both mono- and diesters, while the propylene glycol esterification product was principally monoester, contaminated with considerable low-melting amorphous material.

Further preliminary experiments were carried out with ethylene glycol in order to reduce the amount of diester formed and of the inner esters and polymerization products produced by this method of esterification. The amount of catalyst was reduced to 0.5%, the amount of glycol used was increased to 10 moles per mole of acid, and in several cases toluene was replaced by xylene as the reaction medium. The effect of time of refluxing was also determined. The results are summarized in Table I.

Glycol Monoesters.—The method of esterification giving the best yield of most nearly pure ethylene glycol mono-12-hydroxystearate was as follows:

1. Ethylene glycol mono-12-hydroxystearate, $CH_3OHCH_2OCO\,(CH_2)_{10}\,CHOH\,(CH_2)_5\,CH_3$.—Fifty grams of 12-hydroxystearic acid ($^1/_6$ mole) was placed in a 500-cc., round-bottom flask with 50 Gm. of xylene, 103.5 Gm. ethylene glycol ($^{10}/_6$ mole) and 0.25 Gm. toluene *p*-sulfonic acid (0.5% of weight of 12-hydroxystearic acid). The flask was fitted with a moisture trap and reflux condenser and heated in an oil bath at 160° C., refluxing for 30 min. at that temperature. During this time, 3.45 cc. of aqueous layer separated, of which approximately 55% was ethylene glycol. The xylene was removed completely under reduced pressure on a water bath, and the residue transferred to a separatory funnel with 500 cc. of ether. The excess glycol (about 75 cc.) was drawn off, diluted with about 150 cc. H_2O and washed 3 times with 50-cc. portions of ether to remove any ester dissolved by the glycol. The ether washings were added to the main ether solution and the whole washed thoroughly, first with 10% aqueous K_2CO_3 and then with water. The ether solution was then dried over anhydrous Na_2SO_4 and the solvent removed completely. Yield of crude crystalline ester, 49.8 Gm. (86.8% of theory for monoester); m. p., 67.0–68.8° C.; hydroxyl value, 311.2 (theoretical for monoester, 325.9; for diester 178.9).

A purer product which melted at 67–68.5° C., having an hydroxyl value of 318, was obtained by fractional crystallization.

2. Propylene glycol mono-12-hydroxystearate $CH_3CHOHCH_2OCO\,(CH_2)_{10}\,CHOH\,(CH_2)_5\,CH_3$.—Fifty grams of 12-hydroxystearic acid ($^1/_6$ mole) was esterified in a similar manner with 127 Gm. propylene glycol ($^{10}/_6$ mole), 0.25 Gm. toluene *p*-sulfonic acid (0.5%) and 50 Gm. xylene. After 1 hr. at 160° C., 4.30 cc. of aqueous layer separated in the moisture trap (approximately 27% glycol as calculated from refractive index). After removing the solvent and working up the reaction product as previously, the yield of crude ester obtained was 58.7 Gm. (98.1% of theory for monoester), having no sharp melting point, hydroxyl value 296.1 (calculated for monoester, 313). Upon one recrystallization of a portion from acetone, the pure ester was obtained in large, irregularly shaped crystals forming rosettes, which melted at 63–66° C. and had an hydroxyl value of 316.

3. Trimethyleneglycol mono-12-hydroxystearate $CH_2OHCH_2CH_2OCO\,(CH_2)_{10}\,CHOH\,(CH_2)_5\,CH_3$.—Thirty grams of 12-hydroxystearic acid ($^1/_{10}$ mole) was esterified as previously with 76.1 Gm. trimethylene glycol ($^{10}/_{10}$ mole) using 0.15 Gm. toluene *p*-sulfonic acid (0.5% catalyst) in 60 Gm. xylene. In 1 hr. at 160° C. (bath temperature) 3.55 cc. aqueous layer separated (49% glycol). After evaporation of xylene under reduced pressure and removal of excess glycol and unesterified acid, 34.5 Gm. of crude ester (96.1% of theory for monoester) was obtained, melting at 56–60° C. Hydroxyl value was found to be 300.5 (calculated for monoester, 313). A first crop obtained from one crystallization from acetone melted at 60.5–62° C. and had an hydroxyl number of 308.

Glycol Diesters.—In the preliminary experiments to determine the optimum conditions for the forma-

TABLE II.—MELTING POINTS OF THE GLYCOL ESTERS OF 12-HYDROXYSTEARIC ACID

Compound	Mol. Wt.	Hydroxyl Value Found	Hydroxyl Value Calcd.	M. P., ° C.
Ethylene Glycol Monoester	344.3	318	325.9	67–68.5
Propylene Glycol Monoester	358.3	316	313.1	63–66
Trimethylene Glycol Monoester	358.3	308	313.1	60.5–62
Ethylene Glycol Diester	626.6	178	179.1	90–92
Trimethylene Glycol Diester	640.6	176	175.1	81.3–82.5

tion of monoesters small amounts of the diesters of ethylene glycol and trimethylene glycol were obtained upon fractional crystallization of the esterification products of these glycols. No indication of the formation of propylene glycol diester was encountered.

1. Ethylene glycol di-12-hydroxystearate:

$$\begin{array}{l} CH_2OCO(CH_2)_{10}CHOH(CH_2)_5CH_3 \\ | \\ CH_2OCO(CH_2)_{10}CHOH(CH_2)_5CH_3 \end{array}$$

Upon fractional crystallization of the product obtained from the esterification of 100 Gm. of 12-hydroxystearic acid (1/3 mole) with 42 Gm. ethylene glycol (2/3 mole), 12.8 Gm. of diester was obtained as a hard brittle noncrystalline material melting at 90–92° C. and hydroxyl value 177.6 (theoretical for ethylene glycol-12-hydroxystearate, 178.9).

2. Trimethylene glycol di-12-hydroxystearate:

$$\begin{array}{l} CH_2OCO(CH_2)_{10}CHOH(CH_2)_5CH_3 \\ | \\ CH_2 \\ | \\ CH_2OCO(CH_2)_{10}CHOH(CH_2)_5CH_3 \end{array}$$

Fractional crystallization of the esterification product from 50 Gm. 12-hydroxystearic acid (1/6 mole) and 38 Gm. trimethylene glycol (2/6 mole) yielded 6.7 Gm. of crystalline material melting at 82–83° C. and having an hydroxyl value of 176 (theoretical for trimethylene glycol di-12-hydroxystearate, 175.1).

PROPERTIES AND APPLICATIONS

Melting Points and Solubilities.—The melting points of the homologous glycol monoesters are quite close, the isomeric propylene and trimethylene glycol esters being almost identical in melting point. The diesters prepared melt at temperatures considerably higher than the monoesters.

In structure, all these esters, with the exception of ethylene glycol diester, are markedly crystalline when pure. The ethylene glycol diester is apparently amorphous even after repeated recrystallization.

The monoesters are freely soluble in alcohol, ether, acetone and similar organic solvents, but only sparingly soluble in the hydrocarbon solvents even when hot. On the other hand, the diesters, while less soluble in the hydroxylated organic solvents, are miscible in hot hydrocarbons. Upon cooling such mixtures of ethylene glycol diester with hydrocarbons, gels are formed whose firmness and opacity depend on the amount of diester present. When the solvent is removed from such a gel of ethylene glycol diester with a volatile hydrocarbon, the diester is obtained as a hard, brittle noncrystalline material, resembling a high-melting wax of the carnauba type.

The pure glycol esters are not water-dispersible. However, they may be made readily dispersible by admixture of small amounts of soap or other surface-active agent. Such self-dispersing mixtures are prepared by melting together the appropriate glycol ester with a definite amount of 12-hydroxystearic acid, adding an amount of tri-isopropanolamine or other alkali sufficient to neutralize all or some part of the free acid, and mixing thoroughly before allowing to cool. When such compositions are stirred vigorously with boiling water, stable homogeneous dispersions are obtained upon cooling, varying in consistency, appearance and properties not only with the amount of water used, but also with the amount of soap, free acid and the type of ester present in the self-dispersing composition.

The crystalline character and the sharp melting point of the pure esters are not readily altered by admixture with each other. However, since the pure ethylene glycol di-12-hydroxystearate is noncrystalline, this ester when mixed with an equal weight of monoester forms a hard, brittle, wax-like material with a conchoidal fracture, melting at about 83° C.

In order to determine the applicability of these esters in ointment bases and similar compositions, the compatibilities of the mono- and diesters of ethylene glycol with white petrolatum, cacao butter and soft paraffin (m. p. 48° C.) were investigated.

The addition of monoester to medium-fiber white petrolatum up to 5% was unsatisfactory, since the melted ingredients were not miscible and upon solidification a nonhomogeneous, salve-like semi-solid was obtained. The diester, in the same and higher proportions, produced firm homogeneous gels. When melting points were determined by the U. S. P. method for materials belonging to Class II[1], the mixture of petrolatum with 5% of monoester was observed to rise in the capillary tube at about 58° C., while the mixture with 10% of diester was observed to rise at about 84° C. However, softening was apparent in both at considerably lower temperatures. With cacao butter, similarly, the monoester produced a nonhomogeneous, salve-like semi-solid while the diester formed homogeneous gels. These gels, however, after several days lost their characteristic appearance and became more or less wax-like.

[1] *Cf.* "United States Pharmacopœia XI," p. 455.

Likewise, the monoester composition hardened considerably upon standing. The mixture containing 2% of diester was quite soft, while that containing 5% was fairly firm. These mixtures, as well as the one containing 5% of monoester, softened at about 33–35° C. but required warming to a considerably higher temperature to become clear.

The soft paraffin mixture with 5% monoester was a nonhomogeneous, somewhat plastic semi-solid, while a similar mixture with 5% diester was homogeneous and highly plastic. Both mixtures softened at about the same temperature (48° C.) at which the pure paraffin melted, but were considerably more plastic than the latter at room temperature.

Ointments and Cosmetic Creams.—The glycol esters are not suitable as wax substitutes in beeswax-borax type creams since they are pure crystalline materials containing no free acid and are quite stable to the action of mild alkali under the conditions of preparation of these creams. In the form of self-dispersing compositions, however, they can be used to some extent in vanishing creams as stiffeners provided they are not present in high enough concentration to cause graininess.

Emulsified cosmetic creams of the water-miscible type were prepared from these esters by the use of a surface active agent such as Aerosol OT. Here too, however, the crystalline character of the pure esters causes a grainy appearance in the finished product unless the concentration of ester is carefully selected or a noncrystalline ester-composition is used (*e. g.*, equal parts of ethylene glycol mono- and diesters).

DISCUSSION

These synthetic products may be generally described as solid esters of the nonemulsifying type. From this preliminary study of their properties, they should have considerable interest as wax substitutes in many types of cosmetics, pharmaceuticals and also in industrial products. Their high melting points, unusually high for polyhydric fatty acid esters, their freedom from color and odor, and their good stability suggest application as synthetic waxes with special properties for coatings, insulating materials, polishes and similar industrial materials. The applicability of these glycol esters is further widened by their ready conversion into solid esters of the emulsifying type.

SUMMARY

Methods have been given for the synthesis of the monoesters of 12-hydroxystearic acid with the homologous dihydric alcohols, ethylene glycol, propylene glycol and its isomer, trimethylene glycol. Some physical properties of these esters as well as of the diesters of ethylene glycol and trimethylene glycol, prepared in the course of the development of these methods, have been evaluated and some applications have been suggested.

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A Preliminary Study of Oils of Cassia*

By Roy C. Darlington† and B. V. Christensen‡

Oil of Cassia, one of the oldest drugs (1) in existence, has been one of the most widely adulterated of the drugs official in the United States Pharmacopœia. Although each revision of the U. S. P. has brought forth constructive changes in the monograph of the oil in an attempt to assure the delivery of a high-quality oil to the consumer, these adulterations have continued. Some of the adulterants used have been rosin, alcohol, balsams, and recently synthetic cinnamic aldehyde (2). The detection of the adulteration of a drug is dependent upon, first, knowing the physical characteristics of the authentic drug and, second, upon having a satisfactory method of assay for that drug. In this study an attempt has been made to determine the physical constants and to devise a satisfactory method of assay for an authentic Oil of Cassia.

The materials used in this study consisted of the extracted oil from the barks of *Cin. Cassia*, *Cin. Zeylanicum*, *Cin. Saigonicum* and the technical and U. S. P. oils of *Cin. Cassia* obtained on the market.

* Presented to the Scientific Section of the A. PH. A., Denver Meeting, 1942.

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EXPERIMENTAL

The experimental work involved the extraction of the oil from the barks and determination of physical constants of these oils. The commercial oils were used for experimentation with various methods for total aldehyde assay and methoxy determinations by a modification of the Vieböck and Schwappach method (3).

Before extraction of the barks, they were identified microscopically from prepared slides (4). Both steam distillation and the Clevenger method (5) were used in the extraction of the oils from the barks. The results are shown in Table I.

TABLE I.—EXTRACTION OF OILS

Species of Bark	Method of Extraction	Yield, %
Cassia	Clevenger method	0.855
Zeylanicum	Steam distillation	0.56
Saigonicum	Steam distillation	1.14

The extracted oils were dried over anhydrous sodium sulfate and the following constants determined. The above oils will henceforth be designated as *A*, *B* and *C* in the order given.

TABLE II

Oil	Index of Refraction at 20° C.[a]		Specific Gravity at 25° C.[b]
	Observed	True	
A	1.6080	1.6070	1.0423
B	1.6062	1.6052	1.0457
C	1.6166	1.6156	1.0491

[a] Abbe refractometer correction, −0.001°.
[b] Gay-Lussac specific gravity bottles (1, 2 and 5 Gm.), Arthur H. Thomas Co.

The specific gravity of the extracted Oil of Cassia was found to be slightly less than the specification in the U. S. P. The index of refraction was within the limits of the U. S. P. requirement.

TABLE III.

Oil	Index of Refraction at 20° C.[a]		Specific Gravity at 25° C.[b]	Optical Rotation at 25° C.[c]
	Observed	True		
1	1.6132	1.6122	1.0575	+0.5°
2	1.6091	1.6081	1.0540	−0.5°
3	1.6051	1.6041	1.0504	+0.2°
4	1.6070	1.6060	1.0542	0.0°

[a] Abbe refractometer correction, −0.001°.
[b] Gay-Lussac specific gravity bottle (1, 2 and 5 Gm.), Arthur H. Thomas Co.
[c] Schmidt and Haensch polariscope.

Due to the small quantities of the extracted oils available, it was deemed necessary to conduct some experiments on commercial oils. Hence 4 such oils were used and the constants were first determined in order to check with the U. S. P. requirements. These oils are designated by the numbers 1, 2, 3 and 4 and the constants as found are given in Table III.

The constants of the commercial oils were within the limits of the U. S. P. requirements. Oil 4 was a technical oil and gave a positive test for rosin. Upon the sensitivity of the U. S. P. test for rosin it was found to be sensitive to at least 0.05% of rosin.

ASSAY FOR TOTAL ALDEHYDES

The U. S. P. method of assaying for total aldehydes was found to be unsatisfactory. It was found that approximately 45 cc. of 25% sodium bisulfite was required for neutralization in the assay. This was impossible in a 100-ml. flask and necessitated the use of either only 5 cc. of oil or a flask of greater capacity.

Because of the above facts other methods of assaying for total aldehydes were tried with results as given below:

I. U. S. P. Method Using Saturated solution of Sodium Bisulfite as a Netutralizing Agent.—The amount required for neutralization was reduced, but not enough to term the method satisfactory.

II. U. S. P. Method Using Metabisulfite ($Na_2S_2O_5$) as a Neutralizing Agent (25% and a Saturated Solution).—This method was equally as efficient but no better than the U. S. P. method and therefore unsatisfactory.

III. U. S. P. Method Using Acetic Acid (10% and 36%) as a Neutralizing Agent.—Neutralization was impossible with 10% acetic acid. Neutralization was obtained with 36% acetic acid but the solution in the flask became very alkaline when the flask, after cooling, was filled with a saturated solution of sodium sulfite. It appears that the use of any acid as a neutralizing agent is contraindicated in this assay.

IV. U. S. P. Method Using M/5 Solutions of Sodium Monobasic Phosphate or Boric Acid as Neutralizing Agent.—The assay was impossible, a bright orange precipitate forming in both cases.

V. Total Aldehyde Assay Using 25% Sodium Bisulfite Only.—Fifty cubic centimeters of 25% sodium bisulfite were added to 10 cc. of Oil of Cassia in a cassia flask and shaken until the dense curdly aldehyde compound was formed. The flask was then heated in a boiling water bath for 5 to 6 min. with intermittent shaking until solution of the precipitate was effected. After cooling, the flask was filled into the neck with the same solution and the reading taken after the insoluble portion of the oil separated.

To date, 20 assays on 4 samples of oil have been made and all checked with the results obtained by the U. S. P. method, and all were satisfactory except one (oil 4). With this oil, which tested positive for rosin, an emulsion was formed below the insoluble portion making the reading of the assay difficult. However, this same difficulty was encountered in using the U. S. P. method of assay on this oil. The results of the assays of the various cassia oils by the different methods are given in Table IV. From the results it is seen that the same results were obtained from the various methods used.

METHOXY DETERMINATION OF OIL OF CASSIA

All assays for methoxy content were conducted on commercial cassia oils by the modified Vieböck and Schwappach method (3). The size of the samples ranged from 80 mg. to 300 mg. The results of assays by this method were found to be consistent.

TABLE IV.

Oil Used	Amount Used, Cc.	Method Used	Total Aldehydes, %
1	10	U. S. P.	92
1	5	U. S. P.	92
1	10	Sat. $NaHSO_3$[a]	92
1	10	Sat. metabisulfite[a]	92
1	10	Acetic acid, 36%[a]	92
1	10	25% $NaHSO_3$ only	92
1	5	25% $NaHSO_3$ only	92
2	10	U. S. P.	84
2	5	U. S. P.	84
2	10	Sat. $NaHSO_3$[a]	84
2	10	Sat. metabisulfite[a]	84
2	10	Acetic acid[a]	84
2	10	25% $NaHSO_3$ only	84
2	5	25% $NaHSO_3$ only	84
3	10	U. S. P.	86
3	5	U. S. P.	86
3	10	Sat. $NaHSO_3$[a]	86
3	10	Sat. metabisulfite[a]	86
3	10	Acetic acid, 36%[a]	86
3	10	25% $NaHSO_3$ only	86
3	5	25% $NaHSO_3$ only	86
4	10	U. S. P.[b]	86 (approx.)
4	5	U. S. P.[b]	86 (approx.)
4	10	Sat. $NaHSO_3$[b]	86 (approx.)
4	10	Sat. metabisulfite[b]	86 (approx.)
4	10	Acetic acid, 36%[b]	86 (approx.)
4	10	25% $NaHSO_3$ only[b]	86 (approx.)
4	5	25% $NaHSO_3$ only[b]	86 (approx.)

[a] Used to neutralize Na_2SO_3 as directed in U. S. P.
[b] Reading difficult due to emulsion formed.

However, the efficiency of this method is impaired considerably by the frequent bumping which occurs despite the use of a boiling rod in the reaction flask. In an attempt to correct this fault porcelain chips, glass beads and carborundum were used in the order named. The carborundum was found to be the only one more effective than the boiling rod.

Difficulty is also encountered in the attempt to maintain a steady stream of carbon dioxide through the apparatus due to the building up of pressure in the system. This difficulty might be eliminated by creating a partial vacuum in the system. Lack of time has prevented the trial of this suggested modification.

DISCUSSION

It has long been known that the reaction between an aldehyde and a sulfite or bisulfite is facilitated by neutrality and is reversed by acid and alkali. Because of this, the use of acetic acid is contraindicated as a neutralizing agent, a neutral solution being difficult to obtain because of the hydrolysis of the sodium acetate formed in the neutralization.

It is also known that to facilitate the completion of a reaction the reactants must be brought into intimate contact with each other. This cannot be accomplished in an overly filled flask and hence negates the use of the bisulfite or metabisulfite solution as a neutralizing agent in the sulfite method of assay.

The use of 25% sodium bisulfite only, in assaying for total aldehydes, appears to be the most basically sound method as yet tried. It is seen that the end product of the reactants is identical with that of the U. S. P. method, the difference being that in the U. S. P. method 2 molecules of sodium hydroxide are formed while in the bisulfite method only the stable sulfonate is formed, and the solution in the reaction flask can only approach neutrality as the reaction proceeds, thus facilitating completion of the reaction.

Similarly by considering the reactions occurring during the neutralization in the U. S. P. method of assay a logical explanation of the large volume of sodium bisulfite required is readily obtained.

SUMMARY AND CONCLUSIONS

1. A study of the physical properties of extracted oils from 3 species of cinnamon bark and 4 commercial oils was made.

2. The results of the assay of the commercial oils for total aldehyde content by the U. S. P. and other methods are given and discussed.

3. The modified Vieböck and Schwappach method of assay for methoxy group is discussed.

4. In the assay of Oil of Cassia for total aldehyde content the method using 25% sodium bisulfite solution only appears to be more efficient than other methods studied to date.

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